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Cationic lysine uptake by System R^+ and zwitterionic lysine uptake by System B in brush border membrane vesicles from larval *Manduca sexta* midgut

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Abstract

Lysine uptake was studied at pH 7.4 and 10.0 by rapid filtration methods in brush border membrane vesicles from fifth instar larvae of a model insect, the tobacco hornworm, *Manduca sexta (Lepidoptera, sphingidae)*. At both pH values the uptake was mediated by K^+ coupled symport. The uptake rate increased between pH 5.5 and 10, especially so in the alkaline range. The total lysine uptake could be divided into two components based upon lysine's ionic form as a function of pH. Lysine uptake at pH 7.4 was strongly *cis*-inhibited by arginine but at pH 10 was *cis*-inhibited and *trans*-stimulated by many neutral amino acids, e.g. leucine, but not by arginine. Lysine uptake by the arginine-inhibitable component paralleled the titration curve of cationic lysine whereas uptake by the leucine-inhibitable component paralleled that of zwitterionic lysine. Evidently, the brush border membrane contains at least two separate, K^+-dependent amino acid symporters (co-transporters) that mediate lysine uptake. A cationic amino acid : K^+ symporter selects cationic lysine and arginine but not histidine and other amino acids. One or more zwitterionic amino acid : K^+ symporters select zwitterionic lysine, possibly arginine, histidine and neutral amino acids. Based upon these substrate repertoires, the zwitterionic symporters are B-type systems whereas the cationic symporter is identical with System R^+, which partially resembles System y^+. Arginine uptake in vivo is likely to be mediated mainly by System R^+ whereas lysine uptake is likely to be mediated by System B-type symporters.

Keywords: Lysine : K^+ symport; Co-transport; Arginine; Tobacco hornworm

1. Introduction

The basic amino acids, lysine and arginine, are both essential for growth and must be taken up from the diet [1]. The analysis of their absorption is complex because they change in ionic form within the physiological pH range. Thus they are predominantly cationic at pH 7.4, which is characteristic of mammalian intestine, but are predominantly zwitterionic at pH 10.0, which is characteristic of anterior and middle regions of lepidopteran larval midgut.

In mammalian non-epithelial cells, the cationic forms of lysine, arginine and histidine are transported independently of Na^+ by System y^+, which is a uniporter [2–4]. By contrast, in the brush border of vertebrate intestinal and renal epithelial cells, lysine is transported by a Na^+-dependent co-transport system, which is a symporter [5–8]. In the brush border membrane (BBM) of the midgut epithelial cells in lepidopteran larvae, amino acids are co-transported with K^+, rather than Na^+, as an adaptation to the K^+ rich, plant diet of the larvae [9,10]. A lysine-specific system that rejects arginine has been proposed in *Philosamia cynthia* larvae because arginine did not *trans*-stimulate lysine uptake and because arginine inhibition of lysine uptake was noncompetitive [11,12]. However, a lysine specific system could not be confirmed in *M. sexta*, where lysine *trans*-stimulated and *cis*-inhibited arginine uptake at pH 7.4 [13].

We will show that lysine is taken up by at least two separate systems in BBMV from larval midgut of *M. sexta*: System R^+ accepts only cationic lysine and arginine [13] whereas one or more B-like systems accept many zwitterionic amino acids, including zwitterionic lysine, and perhaps zwitterionic arginine [14]. In the posterior midgut of living larvae at pH 8 [15], lysine absorption by (ca-

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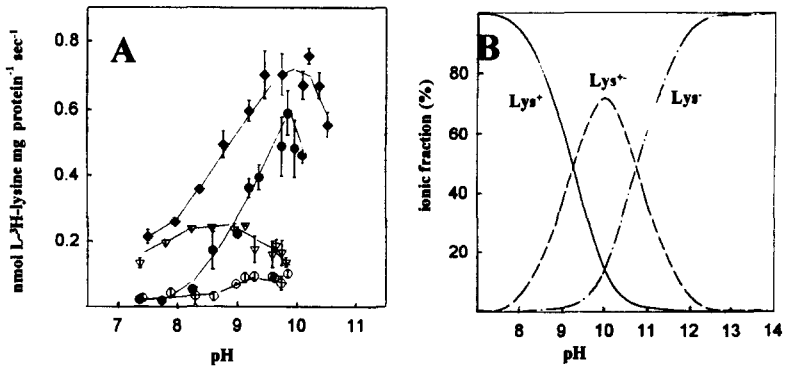

Fig. 1. Effects of pH on lysine uptake and ionic form change. A: pH profile of lysine uptake. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation were: (inside) mannitol 185, Hepes 10, Tris 5, pH 7.4 (outside) mannitol 117, KSCN 50, Tris-MES 30 pH 7.4–8.5, AMPD-MES 30 pH 8.5–9.2, AMPD-MES 30 pH 9.2–10.3, L-[^{3}H]lysine 0.5 only (♦); plus L-arginine 5 (●); plus L-leucine 15 (▽); plus both L-arginine 5 and L-leucine 15 (○). B: relative concentrations of the three ionic forms of lysine as function of pH calculated from lysine pK values of 9.2 of α-amino group and 10.8 of ω-amino group.

tionic) System R^+ would be expected, except that it would be blocked by arginine (Liu and Harvey, unpublished data), whereas in middle and anterior regions at pH > 10, lysine uptake by the (zwitterionic) System B-type symporters would be expected.

2. Materials and methods

2.1. Vesicle preparation and lysine uptake measurements

Methods for preparing brush border membrane vesicles from *M. sexta* larvae by a differential magnesium precipitation method [16,17] and for measuring [^{3}H]lysine uptake by a rapid filtration procedure [18] were described previously [17]. Protein concentrations were determined by the dye-protein binding method [19] using a commercial reagent (Bio-Rad, Richmond, CA) with bovine serum albumin as a standard. Intravesicular and extravesicular component concentrations are given in each figure legend as 'inside' and 'outside' respectively. The initial uptake rate was calculated using SigmaPlot (Jandel Scientific, San Rafael, CA) as the mean slope ± SD (n = 3 or 4) of lines through uptake values 2 and 6 s after mixing.

2.2. Chemical reagents

L-[4,5-^{3}H]lysine and L-[2,5-^{3}H]histidine were from ICN Biochemicals (Costa Mesa, CA) or from Sigma Chemical Co. (St. Louis, MO). Non-radioactive amino acids, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), tris(hydroxymethyl)aminomethane (Tris), carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) and tetramethylammonium hydroxide (TMAOH) were from Sigma. Aminomethylpropanediol (AMPD) was from the Eastman Kodak Company (Rochester, NY). 2-(*N*-morpholino)-ethanesulfonic acid (MES) was from ICN Biochemicals (Cleveland, OH). All other reagents were analytical grade products from either Fisher (Pittsburgh, PA) or Mallinckrodt (St. Louis, MO).

3. Results

3.1. pH dependence of lysine uptake

The initial rate of L-[^{3}H]lysine uptake increased from pH 7.4 to 10 (Fig. 1A) but decreased from pH 7.4 to 5.5 (Fig. 2). Since lysine's α-amino group has a pK_a value of 8.95, the pH dependence in the alkaline range is likely to involve protonation/deprotonation of the substrate (lysine) itself. The observed pH dependence of uptake suggests that cationic and zwitterionic lysine uptakes can be studied

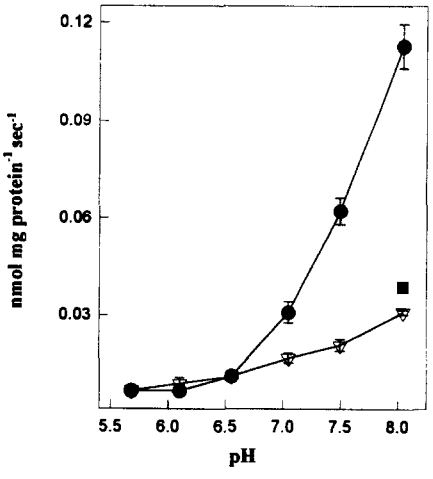

Fig. 2. pH profile of lysine and histidine uptake. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation were: (inside) mannitol 235, Hepes 10, Tris 5 pH 7.4 (outside) mannitol 87, KSCN 50, Mes-Tris 40 pH 5.7–6.5, Hepes-Tris 40 pH 7.0–8.0, L-[^{3}H]lysine 0.04 (●) or L-[^{3}H]histidine 0.04 (▽).

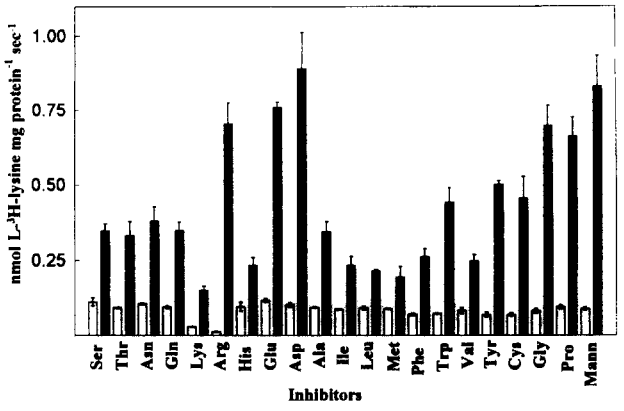

Fig. 3. *Cis*-inhibition of lysine uptake by 20 common amino acids. The initial rates of uptake are shown at pH 7.4 (open bars) and at pH 10.0 (solid bars). The final concentration (mM) of components inside and outside of the vesicle at time zero of incubation were: (inside) mannitol 200, Hepes 90, Tris 45 pH 7.4, or AMPD-HCl 50 pH 10.0 (outside) mannitol 100, KSCN 50, inhibitory amino acid 10.0, Hepes 90, Tris 45, pH 7.4, or AMPD-HCl 50 pH 10.0, L-[^{3}H]lysine 0.5.

almost independently of each other by adjusting the pH to 7.4 and 10.0 respectively.

3.2. *Cis-inhibition of lysine uptake*

Lysine uptake rates were measured separately with each of the 20 naturally occurring amino acids as inhibitors, with an inhibitor: substrate ratio of 20:1 and a 50 mM KSCN gradient, at pH 7.4 and 10.0. At pH 7.4, where lysine is > 90% cationic, its uptake was most strongly *cis*-inhibited by arginine and lysine (Fig. 3). By contrast, at pH 10.0, where lysine is mostly zwitterionic, its uptake was *cis*-inhibited by the majority of neutral amino acids as well as by lysine and histidine, but not by arginine (Fig. 3). Notably, neutral amino acids, such as leucine, isoleucine and methionine, that inhibited lysine uptake strongly at pH 10.0, inhibited it weakly at pH 7.4. Abolishing the salt gradient did not change the inhibition profile (Fig. 4); the profiles with and without a gradient were highly correlated ($r = 0.86$).

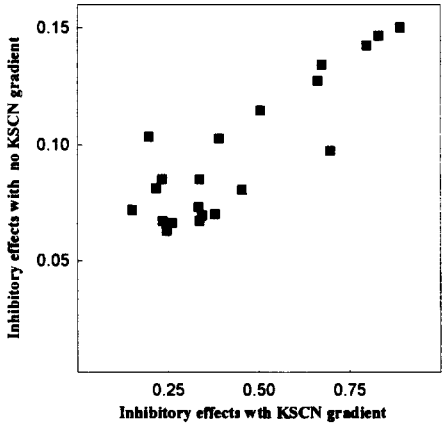

Fig. 4. Correlation between the lysine uptake inhibitions by 20 amino acids with KSCN gradient and that with KSCN but no KSCN gradient. The 20 points in the plot represent lysine initial uptake rates under the inhibition of 20 common amino acids in the conditions specified on the ordinate and abscissa. The experimental conditions with the KSCN gradient at pH 10.0 are specified in Fig. 1. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation with KSCN but no KSCN gradient were: (inside) mannitol 100, KSCN 50, AMPD-HCl 50 pH 10.0 (outside) mannitol 100, KSCN 50, L-[^{3}H]lysine 0.5, each of 20 inhibitory amino acids 10.0, AMPD-HCl 50 pH 10.0. 1 h before the experiment valinomycin was added to the vesicle suspension to yield a final concentration of 8 μg mg^{-1} protein.

3.3. *Trans-stimulation of lysine uptake*

The initial lysine uptake rates were measured in the presence of an outwardly directed gradient of each of the 20 natural amino acids at pH 10.0 (Fig. 5) with the *trans*-membrane voltage clamped at zero. In a typical time course, lysine and leucine elicited lysine accumulation three and four times the equilibrium values, respectively, whereas arginine and mannitol were ineffective (Fig. 6). The *trans*-stimulation (Fig. 5) and *cis*-inhibition (Fig. 3) profiles were similar.

3.4. K^+ *gradient dependence of lysine uptake by both systems*

Lysine uptake was much higher with than without a KSCN gradient at pH 7.4. Thus, in valinomycin treated vesicles, lysine uptake was greater in the presence of KSCN than in mannitol and $TMANO_3$ controls (Fig. 7). Similar results were obtained at pH 10.0 (Fig. 8). These

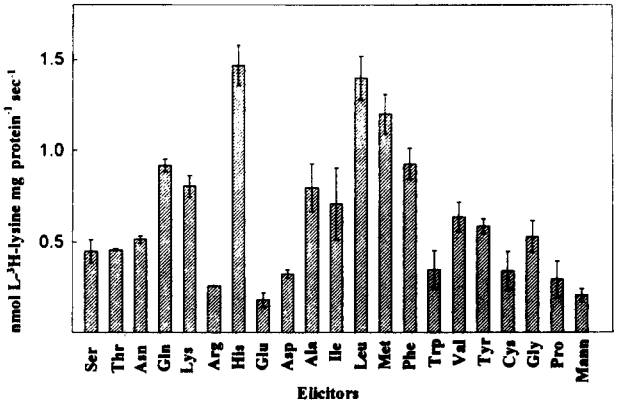

Fig. 5. Initial rate of lysine uptake *trans*-stimulated by 20 common amino acids. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation were: (inside) mannitol 100, KSCN 50, elicitor amino acid 20, AMPD-HCl 50 pH 10.0 (outside) mannitol 125, KSCN 50, elicitor amino acid 1.0, AMPD-HCl 50 pH 10.0, L-[^{3}H]lysine 0.3. 1 h before the experiment valinomycin was added to the vesicle suspension to yield a final concentration of 8 μg mg^{-1} protein.

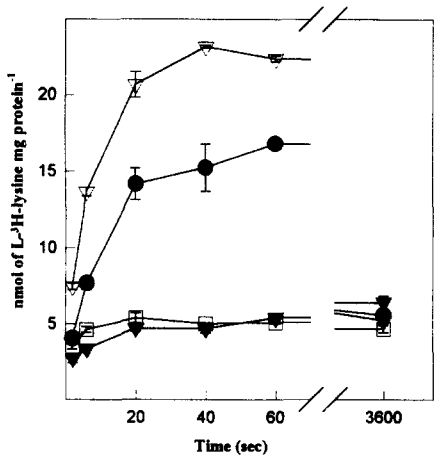

Fig. 6. Initial rate and time course of *trans*-stimulation of lysine uptake by lysine, leucine, arginine and mannitol. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation were: (inside) mannitol 80, KCl 100, AMPD 50, elicitor lysine (●) or leucine (▽) or arginine (▼) or mannitol (□) 40 pH 10.0 (outside) mannitol 120, KCl 100, AMPD-Mes 50 pH 10.0, L-[^{3}H]lysine 1.0, elicitor 2. 1 h before the experiment valinomycin was added to the vesicle suspension to yield a final concentration of 4 μg mg^{-1} protein.

results confirm earlier reports that both the cationic System R^+ [13] and the zwitterionic System B [14,11] are K^+ dependent symporters.

3.5. Is lysine : K^+ symport electrophoretic?

The initial lysine uptake rate and the 1 min accumulation value at pH 10.0 are higher with a K^+ electrochemical gradient (100 mM KSCN) than with a simple K^+ chemical

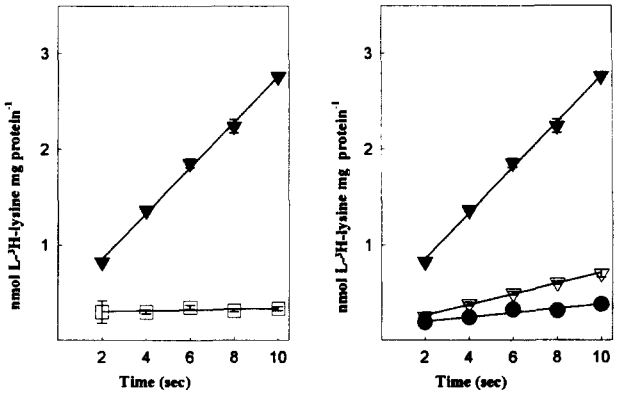

Fig. 7. Initial rates of lysine uptake with and without a cation gradient at pH 7.4. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation under four different conditions were: (1) (inside) mannitol 400, Hepes 50, Tris 25 pH 7.4 (outside) mannitol 200, KSCN 100, Hepes 50, Tris 25 pH 7.4 (▼); (2) the same as (1) except KSCN was replaced by $TMANO_3$ (□); (3) (inside) mannitol 200, KSCN 100, Hepes 50, Tris 25 pH 7.4 (outside) mannitol 200, KSCN 100, Hepes 50, Tris 25 pH 7.4 (▽); (4) (inside) and (outside) were the same: mannitol 400, Hepes 50, Tris 25 pH 7.4 (●). In all cases L-[^{3}H]lysine 0.5 was present outside the vesicles. 1 h before experiment (3) valinomycin was added to the vesicle suspension to yield a final concentration of 4 μg mg^{-1} protein.

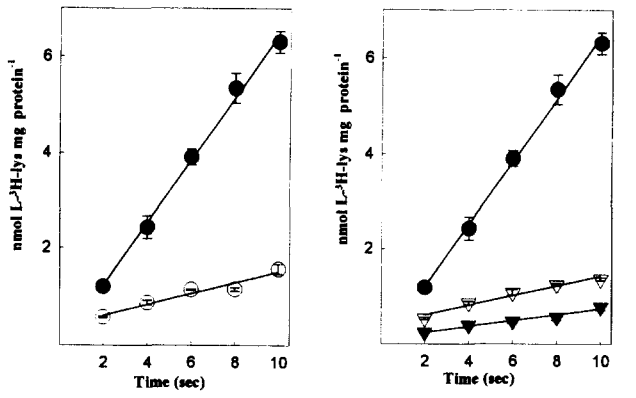

Fig. 8. Initial rates of lysine uptake with and without cation gradient at pH 10.0. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation in four different conditions were: (1) (inside) mannitol 485, Hepes 5, Tris 2.5 pH 7.4 (outside) mannitol 242, KSCN 100, AMPD 50, Hepes 2.5, Tris 1.25 pH 10.0 (●); (2) the same of (1) except KSCN was replaced by $TMANO_3$ (○); (3) (inside) mannitol 285, KSCN 100, Hepes 5, Tris 2.5 pH 7.4 (outside) mannitol 142, KSCN 100, AMPD 50, Hepes 2.5, Tris 1.25 pH 10.0 (▽); (4) (inside) mannitol 485, Hepes 5, Tris 2.5 pH 7.4 (outside) mannitol 435, AMPD 50, Hepes 2.5, Tris 1.25 pH 10.0 (▼). In all cases L-[^{3}H]lysine 0.5 was added outer vesicles. One hour before experiment (3) valinomycin was added to the vesicle suspension to yield a final concentration of 4 μg mg^{-1} protein.

gradient (100 mM KNO_3 outside, 100 mM $TMANO_3$ inside) but without a highly permeant anion gradient (Fig. 9). These results suggest that lysine : K^+ uptake by the zwitterionic symporter may be electrophoretic.

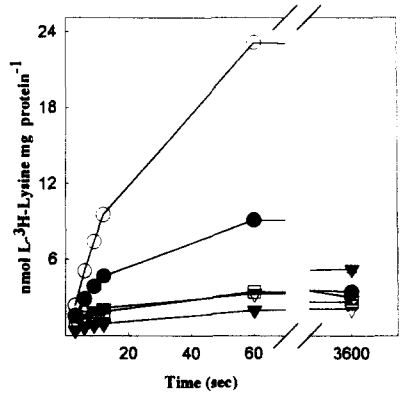

Fig. 9. The enhancement of potassium gradient dependent lysine uptake by highly permeable anion. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation were: (inside) mannitol 400, AMPD 50 (outside) KSCN 100, mannitol 200, AMPD 50 (○); (inside) $TMANO_3$ 200, AMPD 50 (outside) KNO_3 100, $TMANO_3$ 100, AMPD 50 (●); (inside) mannitol 400, AMPD 50 (outside) TMANO3 100, mannitol 200, AMPD 50 (▼); (inside) mannitol 200, AMPD 100 (outside) mannitol 200, AMPD 100, FCCP to a final concentration of 0.1 added to vesicle suspension 30 min before experiment (▽); concentrations inside and outside were the same, mannitol 400, AMPD 50 (□). $pH_i = pH_o = 10.0$ (○), (●), (▼) and (□); $pH_i = 8.0$ and $pH_o = 9.3$ (▽); pHs were adjusted with HCl. In each of the five conditions L-[^{3}H]lysine at 0.5 was present outside of the vesicles.

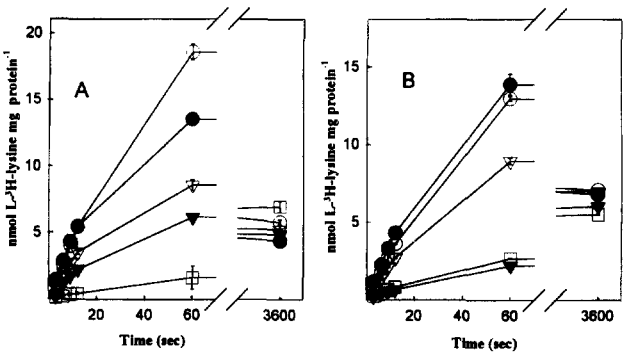

Fig. 10. Cation specificity of lysine uptake. The final concentration (mM) of components inside and outside of the vesicles at time zero of incubation were: (inside) mannitol 400, AMPD 30 (outside) one of five chloride salts, KCl (○), NaCl (●), LiCl (▽), CsCl (▼) or RbCl (□), 100, mannitol 200, AMPD 30, L-[^{3}H]lysine 1.0. pH values were adjusted with HCl to 10.0 (A) and 8.0 (B).

3.6. Cation selectivity of zwitterionic lysine: K^+ symport

At pH 10.0, the alkali ion specificity of lysine uptake is K > Na ≫ Li > Rb > Cs (Fig. 10A), whereas at pH 8.0, the sequence is Na=K > Li ≫ Rb=Cs (Fig. 10B). Thus the cationic selectivity of lysine uptake by the zwitterionic symporter (System B) at pH 10.0 follows a sequence similar to that previously reported for leucine, alanine and phenylalanine uptake by this symporter [14].

4. Discussion

The absorption of cationic, zwitterionic and anionic amino acids is known to be mediated by different transport systems [20,21]. Christensen [20] has shown that this substrate selection is based upon both structure and charge; thus, a change in ionic form of an amino acid with pH is detected by transport systems. Yet most mammalian systems have been investigated only at neutral or acidic pH values. Likewise, despite their adaptation to high alkalinity in vivo (pH 10–12), lepidopteran insect larval midguts have usually been studied at pH 7.4, with a few recent exceptions [14,22–25]. Previously, we have identified a new cationic amino acid:K^+ symporter, System R^+ [13]. We show here that lysine uptake by isolated BBM vesicles at pH 7.4 is mediated mainly by this novel System R^+ but that at pH 10.0 it is mediated mainly by the well known zwitterionic amino acid transporter, System B [26]. The substrate kinetics of lysine and arginine uptake by System R^+ will be compared elsewhere.

4.1. Lysine uptake is not mediated by System y^+

There was little or no lysine uptake at pH 7.4 in the absence of an alkali metal cation (Figs. 7 and 10), suggesting that the Na^+-independent System y^+ is insignificant or absent from midgut BBMV. Moreover, lysine uptake decreased as the pH was lowered from 7.4 to 5.5 (Fig. 2), a range in which System y^+ is pH insensitive [4]. Nevertheless, the substrate spectrum of System R^+ is similar to that of System y^+ [13,5].

4.2. Lysine uptake is mediated by amino acid: K^+ symport and is electrophoretic

The rate of lysine uptake at pH 10 was higher with than without K^+, but was highest in the presence of a KSCN gradient (Fig. 7). Zwitterionic lysine is clearly taken up by symport with K^+. Parallel arguments establish that cationic lysine is also taken up by symport with K^+ (Fig. 7). Na^+-dependent lysine uptake has previously been reported in rat renal brush border membrane vesicles [7]. The stimulation of lysine uptake at pH 10 by a K^+ *electrochemical* gradient (Fig. 9) is important because in vivo there is no K^+ or Na^+ activity gradient to drive amino acid:K^+ symport, which is instead thought to be driven electrophoretically by the large (cytoplasm negative) *trans*-membrane voltage [9].

4.3. The pH dependence of lysine uptake parallels its titration curve

Lysine's initial uptake rate (Fig. 1A) and zwitterionic content (Fig. 1B) both increase from pH 7.4 and reach a maximum at pH 10.0. The non-zero rate of lysine uptake at pH 7.4, where the concentration of zwitterionic lysine is close to zero, can be eliminated with 5 mM arginine; evidently, the two curves become congruent upon suppression of the cationic uptake component (Fig. 1A and Fig. 1B). By adding 15 mM leucine to eliminate the component mediated by the zwitterionic symporter, the peak in uptake activity that is congruent with the peak in the lysine zwitterion titration curve is eliminated; the remaining uptake activity deceases from pH 8 to 10.0 (Fig. 1A) paralleling the decrease of cationic lysine from 100% at pH 7.5 to 15% at pH 10.0 (Fig. 1B). When arginine and leucine were added together, uptake was completely blocked. The pH value at the crossover point of the leucine- and arginine-inhibitable components (Fig. 1A) is quite close to the dissociation constant (pK 8.95) of the α-amino group of lysine.

4.4. The cationic lysine: K^+ symporter is identical with System R^+

Since the cationic lysine:K^+ symporter described here recognizes the cationic forms of lysine and arginine but not histidine, it appears to be identical with System R^+ that we identified previously [13]. We found no evidence for a lysine specific symporter such as that reported in *P. cynthia* [11,12]. Instead, the *P. cynthia* system also appears to be identical with System R^+.

4.5. The zwitterionic lysine: K^+ symporter is identical with the insect System B

The *cis*-inhibition data (Fig. 3) at pH 10.0 suggest strong interaction between zwitterionic forms of neutral amino acids and zwitterionic lysine. This interaction might be an electric or energetic effect of a dissipating alkali metal ion gradient [28]. However, the inhibitory effects of zwitterionic amino acids at pH 10.0 on lysine uptake were similar whether or not the KSCN gradient was present (Figs. 3 and 4). Moreover, the same set of zwitterionic amino acids that were effective as *cis*-inhibitors were also effective as *trans*-elicitors (Figs. 3 and 5). The *cis*-inhibition effects appear to be mainly the result of competition for binding sites on transporters rather than the result of competition for the K^+ gradient. The rejection of zwitterionic arginine at pH 10 by System B [14] contrasts sharply with the acceptance of zwitterionic lysine and most other zwitterionic amino acids by this system (Fig. 3).

4.6. Lysine may be absorbed by System B and arginine by System R^+, in vivo

Although cationic lysine is taken up in BBMV at neutral pH by System R^+ in the absence of arginine (Figs. 1 and 3), System R^+ is not likely to mediate lysine absorption in vivo, where it would be inhibited non-competitively by arginine [[12]; Liu and Harvey, unpublished data] since the concentration of the two basic amino acids are similar in vivo [27]. On the other hand, since zwitterionic arginine interacts weakly with System B in BBMV [13], this system is unlikely to mediate arginine uptake in vivo. These results from BBMV suggest that in anterior and middle midgut regions of feeding larvae, where the pH is > 10, the low cationic arginine concentration would restrict arginine absorption. But arginine absorption would be favored in the posterior midgut of feeding larvae, where the pH is ≈ 8. By contrast, lysine absorption would be impeded at the lower pH in posterior midgut of feeding larvae but would be facilitated by the high pH of anterior and middle midgut of feeding larvae.

4.7. System R^+ rejects cationic histidine

A useful diagnostic feature of System R^+ is its failure to recognize cationic histidine. Thus, histidine *cis*-inhibited and *trans*-stimulated zwitterionic lysine uptake but did not inhibit cationic lysine uptake (Figs. 3 and 5). Labeled histidine uptake activity rises sharply from pH 6.5 to 8.0, whereas the proportion of histidine's cationic form is falling during this pH change (Fig. 2). Finally, histidine uptake is *cis*-inhibited and *trans*-stimulated by the majority of neutral (zwitterionic) amino acids at pH 10.0 (data not shown). Apparently, histidine uptake is mediated mainly by the zwitterionic System B rather than by System R^+.

5. Conclusions

In BBMV from larval lepidopteran midgut, lysine uptake is mediated by at least two amino acid: K^+ symport systems. At neutral pH, cationic lysine and arginine appear to be the only natural substrates for System R^+. At high pH, the zwitterionic forms of lysine, perhaps arginine, histidine and most neutral amino acids are all natural substrates for System B. However, in vivo, arginine would be expected to inhibit lysine uptake by System R^+ non-competitively. Therefore, arginine may be the only substrate in vivo for System R^+ and lysine may be taken up only by System B.

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